cagA Status and Eradication Treatment Outcome of Anti-*Helicobacter pylori* Triple Therapies in Patients with Nonulcer Dyspepsia

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The differences in eradication rates reported in clinical trials aiming to cure *Helicobacter pylori* infection cannot be entirely explained by the type of regimen, bacterial resistance, or lack of compliance. Using data from a clinical trial, a logistic regression model was constructed to determine whether cagA status, assessed by PCR, affects the outcome of eradication. Resistance to clarithromycin (10% of the strains) predicted failure perfectly. In the model (n = 156), a cagA-lacking strain (odds ratio [OR] = 2.2; 95% confidence interval [CI], 1.1 to 4.7), tobacco smoking OR = 3.1; 95% CI, 1.3 to 7.0), and a double dose of proton pump inhibitor in the treatment regimen (OR = 0.3; 95% CI, 0.2 to 0.7) were associated with the treatment outcome. The exact role of cagA in the outcome of *H. pylori* eradication therapy has not been explored. However, the type of histological lesions which it causes in the gastric mucosa may be implicated. Regardless of the mechanism involved, cagA status is a good predictive marker of eradication outcome.

Triple therapies used for the eradication of *Helicobacter pylori* generally include two antibiotics, i.e., clarithromycin and metronidazole or clarithromycin and amoxicillin, and a proton pump inhibitor. In several European multicenter studies, cure rates from 80 to 95% have been obtained using omeprazole (19), lansoprazole (25), or pantoprazole (11), except in France, where the cure rate varied from 70 to 80% (6).

These large multicenter studies have been performed in northern Europe where compliance is better and where resistance of *H. pylori* to antibiotics is lower than in Mediterranean countries (23). However, these European studies included exclusively (11, 19) or essentially (25) peptic ulcer disease (PUD) patients, while a large number of patients with nonulcer dyspepsia (NUD) were included in the French studies. Better eradication rates have been reported in PUD patients than in NUD patients, 73 versus 55%, respectively (P = 0.016) (29). A recent meta-analysis also indicated a better efficacy of these triple therapies in PUD patients than in NUD patients (eradication rates of 90.4 and 77.7% respectively [P = 0.001]) (15).

The cagA gene has been found more frequently in strains from PUD patients than in strains from NUD patients (12, 17, 30). The cagA gene is a marker for the cag pathogenicity island, which is associated with an increased inflammatory response at the gastric mucosal level (1, 10) and severe gastric disease (3, 4). Furthermore, the function of the protein produced by this gene has recently been determined by Stein et al. and Covacci et al. (8, 26).

The question of whether to eradicate *H. pylori* in NUD patients is still debated; therefore, it is interesting to consider the genotype of *H. pylori* strains when evaluating treatment outcome (5, 22, 27, 28). Although limited by a small sample size, one study has provided promising results on the subject (30). For answering the question, the most practical alternative is to consider clinical trials performed on NUD patients. The main advantages of clinical trials, despite their lack of representativity, are the quality of follow-up, data collection, and methodology.

Therefore, the following analysis was conducted to determine the factors involved in the outcome of eradication treatment, particular the cagA status of the *H. pylori* strain harbored. The data used came from a large multicenter clinical trial on *H. pylori* eradication, carried out on NUD patients (18), evaluating a 7-day triple therapy currently recommended in France and Europe (21, 32).

MATERIALS AND METHODS

Study data. The data were issued from a clinical trial carried out by the Aquitaine Gastro Association in southwest France, whose primary aim was to compare two different doses of proton pump inhibitor in a triple therapy for *H. pylori* eradication.

This multicenter, randomized, double-blind trial was conducted on patients with NUD, confirmed by endoscopy, with or without a history of past ulcers. The two arms of treatment were: amoxicillin (1 g twice a day b.i.d.), clarithromycin (500 mg b.i.d.), and pantoprazole (40 mg once a day (o.d.)) versus amoxicillin (1 g b.i.d.), clarithromycin (500 mg b.i.d.), and pantoprazole (40 mg b.i.d.). *H. pylori* status was assessed at inclusion by Campylobacter-like organism test and histology or culture and at 4 weeks after the end of treatment by histology and culture or urea breath test if the patient refused the posttreatment endoscopy.

In this trial, a total of 203 patients were randomized, 192 were included in the intention to treat analysis and finally 166 patients were included in the per protocol (PP) analysis (18). The description of the 37 patients excluded from the trial and the results of the clinical trial have been published (18).

Study population. The present analysis included the PP population of the above-mentioned trial, for whom the cagA gene status of the *H. pylori* strain was available. This population was chosen because the patients had indeed received a treatment which had or had not been successful.

*H. pylori* culture and resistance tests. Culture of *H. pylori* was performed on selective and nonselective media (24) before treatment and 4 to 6 weeks after the end of treatment. The MICs of clarithromycin for *H. pylori* were determined by E-test.
A logistic regression model was constructed using information obtained at inclusion of the patients in the clinical trial as variables and the H. pylori status evaluated at the end of the trial as the outcome measure. The variables used concerned (i) the host: age, gender, body mass index (BMI) (considered to be normal for women between 20.2 and 26.6 kg/m² and for men between 21.6 and 28.2 kg/m² and abnormal otherwise), ethnic origin (Caucasian or others, including Mediterranean, black, and Asian), tobacco consumption (yes or no), alcohol consumption (yes or no), compliance (100% or lower) and presence of H. pylori infection, (ii) the strain: cagA status, clarithromycin susceptibility, and resistance to amoxicillin, tetracycline, and co-trimoxazole, and (iii) the treatment received by the patient during the trial: pantoprazole o.d. versus pantoprazole b.i.d.

**Statistical analysis.** The present analysis of the clinical trial database was performed in order to identify variables which were predictive or linked to the success or failure of H. pylori eradication therapy, in particular, the cagA status of the strain.

A backward elimination procedure was then performed to determine variables related to the host and the strains, in particular, the cagA status of the strain.

**RESULTS**

**Sample.** Among 166 patients included in the PP analysis of the initial trial, 156 patients for whom the H. pylori strains were available were included in the analysis of the present study. Strains from three patients could not be subcultured after initial culture, and in seven cases strains could not be recovered after thawing.

Among the 156 patients, 80 were male (51.3%), and the mean age was 51.7 years (range, 20 to 75 years; standard deviation, 14 years). The mean age of men and women was 49 and 54 years, respectively (P = 0.38). The BMI ranged from 17.4 to 38.2 kg/m²; the mean BMI among men was 25.4 and that among women was 24.8 (P = 0.35). There were significantly fewer smokers among women (12 of 76 [15.8%]) than among men (25 of 80 [31.3%]) (P = 0.02). Concerning the outcome, treatment was successful in 109 patients (69.9%).

Sixteen of 156 strains were clarithromycin resistant (10.2%). Seventy-four of 156 strains (47.4%) were cagA positive using the first set of primers. Among the remaining 82 strains negative for cagA, 10 (12.2%) were positive using the second set of primers; i.e., in total, 84 of 156 (53.8%) strains were cagA positive. There was no difference in the proportion of cagA-positive strains among the clarithromycin-resistant (8 of 16 [50%]) and -susceptible (76 of 140 [54.3%]) strains (P = 0.73).

**Relationship between cagA status and strain and eradication outcome.** The univariate analysis (Table 2) showed that among the variable linked to eradication outcome at a sufficient level to be included in the regression model (P ≤ 0.25), two variables were associated with success—a double dose of pantoprazole versus a single dose and a BMI superior to the normal—and four variables were associated with failure: infection with a cagA-lacking strain, older age, tobacco smoking, and an ethnic origin other than Caucasian. H. pylori was not successfully eradicated in any of the patients with clarithromycin-resistant strains. Among the other variables tested, such as gender and alcohol consumption, no association with eradication outcome was found.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total sample</th>
<th>Eradication failure</th>
<th>OR for failure</th>
<th>95% CI</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o.d.</td>
<td>80 51.3</td>
<td>32 40.0</td>
<td>1.00</td>
<td></td>
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<tr>
<td>b.i.d.</td>
<td>76 48.7</td>
<td>15 19.7</td>
<td>0.37</td>
<td>0.18–0.76</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>109 69.9</td>
<td>36 33.0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior to normal</td>
<td>20 12.8</td>
<td>6 30.0</td>
<td>0.87</td>
<td>0.31–2.45</td>
<td>0.791</td>
</tr>
<tr>
<td>Superior to normal</td>
<td>27 17.3</td>
<td>5 18.5</td>
<td>0.46</td>
<td>0.16–1.32</td>
<td>0.148</td>
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<tr>
<td>Susceptibility to clarithromycin</td>
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<td></td>
<td></td>
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<tr>
<td>Susceptible</td>
<td>140 89.7</td>
<td>31 22.1</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>16 10.3</td>
<td>16 0.00</td>
<td>0.46</td>
<td>0.16–1.32</td>
<td>0.148</td>
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<td>cagA status of strain</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cagA+</td>
<td>84 53.8</td>
<td>20 23.8</td>
<td>1.00</td>
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<tr>
<td>cagA-lacking</td>
<td>72 46.2</td>
<td>27 37.5</td>
<td>1.92</td>
<td>0.96–3.40</td>
<td>0.065</td>
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<td>Ethnic origin of patient</td>
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<tr>
<td>Caucasian</td>
<td>134 86.5</td>
<td>38 28.4</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>21 13.5</td>
<td>9 42.9</td>
<td>1.89</td>
<td>0.66–5.38</td>
<td>0.18</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Non-smoker</td>
<td>119 76.3</td>
<td>30 25.2</td>
<td>1.00</td>
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<tr>
<td>Smoker</td>
<td>37 23.9</td>
<td>17 45.9</td>
<td>2.52</td>
<td>1.16–5.45</td>
<td>0.018</td>
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<td>Age (continuous variable)</td>
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</tr>
<tr>
<td>0.98</td>
<td>0.95–1.0</td>
<td>0.069</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a Results of univariate analysis.

b P not significant if P >0.25 in univariate analysis.

c Mean for total sample, 51.7 years; mean for eradication, failure, 48.5 years.

d NA, not applicable.
There are other examples of infectious diseases, such as meningitis and prostatitis, for which this is the case. Another possible explanation may be related to the fact that cagA-positive strains grow faster than cagA-lacking strains. This point has not been extensively studied but is reported by two authors (7, 31). Mutants in the cag pathogenicity island obtained by Censini et al. exhibited a lower growth rate. Since antibiotics are active during cell division, they are more active on rapidly growing bacteria than on bacteria in stationary phase. Furthermore, the growth rate may influence the density, which is higher in the case of cagA-positive strains, and indirectly the inflammation (2, 12). The role of the cagA gene on eradication outcome may logically be explained by its effect on gastric mucosa. In any case, the cagA gene produces its effect on the outcome and the existing link is undisputable.

Infection with strains resistant to clarithromycin consistently led to treatment failure and therefore could not be included in the model. Indeed, a model cannot be adjusted on a variable for which there are no patients in one category (14), for example, in this case, the category of resistant strains with eradication success. In order to avoid this problem inherent to the methodology, the analysis of cagA status alone was performed using both the entire sample and the subsample of patients harboring susceptible strains to clarithromycin only. The results were similar in both cases. As only 10% of the strains cultured were resistant, the results were valid whether or not these strains were included. Clarithromycin resistance was the strongest predictor of failure, and its global impact will be increasingly important as the prevalence rate of resistance augments. Amoxicillin resistance was not tested because no amoxicillin-resistant strains have yet been detected in France by the National Surveillance Network.

Finally, from a pragmatic viewpoint, it is possible to conclude that the cagA status of H. pylori is a good predictive factor for eradication outcome in NUD patients, independent of resistance status, at the present rate of clarithromycin resistance. This observation leads to two important recommendations. (i) In clinical practice, given the satisfactory correlation between the presence of the cagA gene in the strain and the serological detection of anti-CagA antibodies (9), when H. pylori eradication treatment is considered in NUD patients, it may be helpful in decision making to test for anti-CagA antibodies; a longer treatment may be necessary in CagA-negative patients. (ii) It should be mandatory that results of clinical trials in NUD patients be adjusted on the basis of cagA status of H. pylori strains.

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